POLYANDRY AND FITNESS OF OFFSPRING REARED UNDER VARYING NUTRITIONAL STRESS IN DECORATED CRICKETS

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Abstract.—Females, by mating with more than one male in their lifetime, may reduce their risk of receiving sperm from genetically incompatible sires or increase their prospects of obtaining sperm from genetically superior sires. Although there is evidence of both kinds of genetic benefits in crickets, their relative importance remains unclear, and the extent to which experimentally manipulated levels of polyandry in the laboratory correspond to those that occur in nature remain unknown. We measured lifetime polyandry of free-living female decorated crickets, *Gryllodes sigillatus*, and conducted an experiment to determine whether polyandry leads to an increase in offspring viability. We experimentally manipulated both the levels of polyandry and opportunities for females to select among males, randomly allocating the offspring of experimental females to high-food-stress or low-food-stress regimes to complete their development. Females exhibited a high degree of polyandry, mating on average with more than seven different males during their lifetime and up to as many as 15. Polyandry had no effect on either the developmental time or survival of offspring. However, polyandrous females produced significantly heavier sons than those of monandrous females, although there was no difference in the adult mass of daughters. There was no significant interaction between mating treatment and offspring nutritional regimen in their effects on offspring mass, suggesting that benefits accruing to female polyandry are independent of the environment in which offspring develop. The sex difference in the extent to which male and female offspring benefit via their mother's polyandry may reflect possible differences in the fitness returns from sons and daughters. The larger mass gain shown by sons of polyandrous females probably leads to their increased reproductive success, either because of their increased success in sperm competition or because of their increased life span.

Key words.—Crickets, genetic benefits, *Gryllodes sigillatus*, mate choice, multiple mating, polyandry, sexual selection.

Received March 14, 2002.   Accepted July 3, 2002.

Females of numerous insect species mate more often than is necessary to ensure fertilization of their eggs (Ridley 1988), often at the cost of the increased risk of injury, disease, or predation (Daly 1978). The paradox of female multiple mating is more apparent than real, however, because a recent meta-analysis has revealed that female fitness increases markedly with increased mating rate in insects (Arnqvist and Nilsson 2000). In addition to obtaining sperm with which to fertilize their eggs, females can derive a variety of other direct material benefits by mating more than once: accessory-gland substances that promote increased egg production and oviposition, voluminous ejaculates that yield nutritional benefits following their internal digestion, or nuptial food gifts provided by males at mating (reviewed in Arnqvist and Nilsson 2000).

If the benefits to females of mating more than once have been well established, the advantages to mating with different males (i.e., polyandry) have not. In theory, females could secure the same level of direct benefits through repeated matings with the same male as they could by mating with different males. However, studies across a wide variety of taxa have shown that females are not indifferent with respect to their choice of mates (review in Andersson 1994), and recent work has shown that females may even discriminate against previous mates in favor of novel mating partners (Bateman

1998; Zeh et al. 1998). These observations have led some researchers to suggest that females may derive indirect genetic benefits by mating with more than one male (reviewed in Yasui 1998; Jennions and Petrie 2000; Zeh and Zeh 2001).

Genetic benefits to polyandry can be broadly categorized into two main types, those that lead to an increase in hatching success (short-term effects), as may occur when polyandry lowers the risk of genetic incompatibility of mates (Zeh and Zeh 1997a,b), and those that lead to the increased viability of offspring (long-term effects), as may occur when females selectively fertilize their eggs with sperm of genetically superior sires (Eberhard 1996). The former kind of benefits arise as a result of interactions between parental genomes, whereas the latter arise from qualities intrinsic to paternal genomes (Zeh and Zeh 2001).

Crickets (Orthoptera: Gryllidae) are rapidly becoming a model organism for studies of the evolution of female polyandry, and there is evidence of both short-term (Tregenza and Wedell 1998, 2002; Simmons 2001) and long-term (Simmons 1987) genetic benefits of polyandry in these insects. Surprisingly, however, there are scarcely any published accounts on the degree of polyandry that occurs in free-living females (Rost and Honegger 1987; Souroukis and Murray 1995) and none that provide estimates of polyandry over the life span of females; thus, the extent to which experimentally manipulated levels of polyandry in the laboratory correspond to those that occur in nature remain unknown. Studies of the mating behavior of free-living crickets in outdoor enclosures

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can provide readily obtained, realistic estimates of individual mating frequencies, but the emphasis in previous studies has been on variation in male mating success (e.g., French and Cade 1987; Cade and Cade 1992; Souroukis and Cade 1993).

Laboratory investigations of decorated crickets, *Gryllodes sigillatus*, have revealed that females will mate repeatedly throughout their lives and with many different males when given the opportunity to do so (Burpee and Sakaluk 1993a; S. K. Sakaluk and A.-K. Eggert, unpubl. data). Although direct benefits of multiple matings in this species have been well established (Burpee and Sakaluk 1993b; Ivy et al. 1999), the possible genetic benefits of female polyandry remain unexplored. Female *G. sigillatus* appear ideally suited to such a study because of the opportunities they have for exerting postcopulatory mating preferences (Sakaluk and Eggert 1996; Sakaluk 1997). Females are ideally positioned to determine the fate of their mates' gametes because they can remove and consume the male's externally attached spermatophore any time after mating, and often do so before it has been completely evacuated of sperm (Sakaluk 1984). In *G. sigillatus*, the spermatophore includes a large gelatinous mass, the spermatophylax, that surrounds a smaller sperm-containing ampulla and that is detached and eaten by the female after mating (Sakaluk 1984, 1987). The time required to consume the spermatophylax is linearly related to its mass (Sakaluk 1985), and after it has been completely consumed, the female removes and eats the sperm ampulla (Sakaluk 1984, 1987). Females can greatly influence the paternity of their offspring through their ampulla-removal behavior (Sakaluk 1986; Sakaluk and Eggert 1996); therefore, this behavior provides females with a powerful mechanism of postcopulatory female choice.

Here we provide data on levels of polyandry of free-living females and present the results of an experiment designed to assess long-term indirect genetic benefits of polyandry in *G. sigillatus*. We experimentally manipulated both the levels of polyandry and opportunities for females to select among males, either by presenting multiple males to females sequentially or simultaneously. We employed a repeated-measures design in which full-sib females were replicated across mating treatments; the use of full-sibs means that any variation in offspring viability arising from maternally derived genes should be at least partially mitigated. Because genetic benefits may be manifest only when offspring viability is environmentally challenged, offspring of experimental females were randomly allocated either to a low-food-stress or a high-food-stress regime to complete their development.

MATERIALS AND METHODS

Polyandry in Free-living Females

We obtained data on female mating frequency and degree of polyandry from a study of lifetime mating success of free-living *G. sigillatus*, conducted in a large outdoor enclosure erected in a courtyard at the University of New Mexico, Albuquerque. Despite the seeming artificiality of this habitat, *G. sigillatus* is in fact normally associated with human habitation (Smith and Thomas 1988), and the courtyard already sustained a small population of another synanthropic cricket species, *Acheta domesticus*. The roughly boot-shaped enclo-

sure (approximately 45 m^2) was erected on the concrete surface of the courtyard, and constructed of ¼-in (6-mm) plywood walls (height = 0.61 m) covered with plastic vapor barrier and caulked at the bottom to prevent the escape of experimental individuals. The enclosure, marked off in 1-m^2 quadrats, contained 30 specially designed shelters, more or less uniformly spaced, each equipped with a red filter to permit nighttime viewing of mating activity. Shelters were made of cylindrical PVC couplers (diameter = 12.7 cm; height = 9.5 cm) with four, symmetrically located entrances at the bottom of each shelter to provide a means of access for the crickets; the red filter was seated halfway down the shelter on the inside ridge of the coupler. Each shelter was placed on a ¼-in plywood base (16 cm × 16 cm), and the top of the shelter covered with a glazed ceramic tile (14.3 cm × 13.6 cm), to prevent it from overheating during the day. All shelters were provisioned with food (Purina Cat Chow) and small test tubes filled with water and plugged with cotton wicks. Naturally occurring fissures extending across the surface of the courtyard exposed the underlying soil, which was moistened daily to provide an additional source of water and oviposition substrate. The enclosure was accessible to potential avian predators during the day and to any terrestrial predators that managed to climb into the enclosure, including spiders and skinks; the latter were removed upon their discovery because once inside the enclosure, they had no means of escape.

The crickets used in the study were collected as nymphs in Tucson, Arizona, and brought back to the laboratory to become sexually mature. Crickets were between two and seven days adult age on the day they were introduced into the enclosure. Experimental individuals were marked individually with a numbered plastic tag secured to the pronotum with cyanoacrylic glue. A small amount of fluorescent paint was applied to the hind femora of each individual, so that portable ultraviolet lanterns could be used to quickly locate crickets in the enclosure.

Experimental crickets (25 of each sex) were observed each night for 31 consecutive nights from 2030 h to 0620 h and censused at 20-min intervals; at each census we recorded the location of each cricket, the number of males and females present within the same shelter of a focal individual, and singing activity of males. Between censuses, the enclosure was continuously scanned for the occurrence of copulations. We are confident that we recorded most, if not all, of the copulations occurring in the enclosure during observation periods, because experimental crickets tended to cluster under a few shelters and males remained relatively sedentary. The majority of copulations were directly observed, but some were inferred by the presence of a spermatophore visible at the end of the abdomen of a mated female and the close proximity of a guarding male (see Sakaluk 1991). When an experimental individual was found dead or missing, it was replaced by another cricket of approximately the same age, sex, and mating experience; thus, the sex ratio and density of crickets remained constant throughout the study.

Benefits of Polyandry

Experimental *G. sigillatus* were descendants of approximately 200 adult crickets collected in Tucson, Arizona, in

October 1995. Adult crickets were housed in 75-L terraria, and provisioned with Flukers cricket chow (Baton Rouge, LA), water supplied in 40-ml plastic tissue-culture flasks (NunClon, Rochester, NY) plugged with dental cotton rolls, and egg cartons to provide shelter and to increase surface area. Oviposition substrate, a mixture of sand and vermiculite (1:5), was also provided in plastic containers of various sizes and moistened daily. Nymphs were mass-reared in plastic shoe boxes (16.5 × 30.5 × 8.5 cm) at 28°C on a 12:12 h light:dark cycle and given ample food, water, and portions of egg carton.

To obtain full-sib experimental subjects, newly molted virgin male and female crickets were randomly selected from stock cultures and paired for life, and their offspring reared as above. Newly eclosed adult females emerging from individual sibships were collected daily and housed individually in small plastic containers (10.5 × 10.5 × 9.5 cm). We established triads of full-sib females in which each female was weighed and randomly assigned to one of three mating treatments on the fourth day following her eclosion: (1) the female was housed with a single, randomly selected male for five consecutive days (no-choice treatment); (2) the female was housed with a different male on each of five consecutive days (limited-choice [choice 1] treatment); (3) the female was housed with five males for five consecutive days (full-choice [choice 5] treatment). Thus, females in the no-choice and limited-choice treatments each had access to only one male at a time, but the number of prospective mating partners varied over the five-day period, whereas limited-choice females and full-choice females each had the same number of prospective mating partners, but the number of males to which they had access at any given time varied; all females had the same time period (five days) within which to mate.

After the five-day mating period, males were removed from containers and each female was provided with a plastic weigh boat filled with oviposition substrate that was moistened daily. A sample of eggs was collected from each female and weighed to control for the possibility that females given unlimited opportunities to choose their mates differentially allocate greater resources to their eggs than do females with limited or no choice of mates. Differential reproductive effort of females across treatments could confound any comparison of offspring viability (de Lope and Møller 1993; Petrie 1994). Five eggs were randomly selected from the oviposition dish of each experimental female five days after males had been removed. Eggs were dried at 65°C in a drying oven for 48 h, and weighed to the nearest 1 μg using a Cahn, Beverly, MA, C-31 microbalance.

Experimental females were held in their containers for 28 days, after which they were returned to the breeding colony. Oviposition dishes were maintained until hatching had ceased for at least one week. Nymphs hatching in each experimental container were removed with a mouth aspirator and housed together in a plastic shoebox, where they were initially provided with food and water ad libitum. When the total number of nymphs emerging from an experimental container reached 50, nymphs were randomly separated into two groups of 25 crickets each. One group was provided with cricket chow ad libitum (ad libitum group), whereas the other group was deprived of food every other day (food stress group). Offspring

were reared in an environmental chamber maintained at 32°C on a 12:12 h light:dark cycle. As the offspring approached adulthood, boxes were checked daily for adult eclosion. Dates of adult emergence were recorded, as were the sex and mass of individual offspring.

Treatment effects were assessed with respect to three measures of offspring fitness: developmental time, survival to adulthood, and mass at eclosion. Survival was measured as the percentage of nymphs that attained adulthood. Developmental time was measured in two ways: (1) the time from hatching until the first offspring had undergone adult eclosion; and (2) the time from hatching until 50% of all surviving offspring had undergone adult eclosion (after Simmons 1987). Because decorated crickets exhibit a sexual-size dimorphism, with females being larger than males (S. K. Sakaluk, unpubl. data), the mass of offspring was measured separately for the sexes; offspring mass was measured at adult eclosion because it is a reliable predictor of adult body size at this stage of development (Gray 1997).

To examine the effect of female polyandry and offspring rearing environment on offspring fitness, we employed a randomized-block ANOVA with triad as a blocking factor throughout all analyses and female mating treatment and offspring nutritional regimen as within-block factors. Analyses were conducted using the PROC GLM module of SAS (SAS Institute 2000), following the recommendations of Cody and Smith (1997). Because this kind of analysis requires a balanced experimental design, we eliminated from further analysis any triads in which one or more females died without reproducing or producing fewer than the required 50 nymphs or those in which a female's assigned mate died during the mating period; although failure of a female to reproduce could be due as much to genetic incompatibility as to her own intrinsic sterility, our focus in this study was on offspring fitness per se. A total of 24 complete triads yielded data on reproductive output for 72 females, from which we reared 144 sibships (two per female under two different nutritional regimens).

RESULTS

Mating Frequency of Free-living Females

Data on cricket movements, activity budgets, and selection intensities will be presented elsewhere, but the following three observations are pertinent to the present study: (1) even when given a preponderance of shelters, individuals of both sexes typically aggregated in large clusters in two or three shelters; (2) individuals of both sexes remained in shelters throughout the day; and (3) during the night males spent most of their time within shelters, whereas females spent more time outside of shelters, usually ovipositing (females spend 2–3 h ovipositing nightly; see also Sharma 1976).

We obtained data on more than 350 matings over the course of 31 consecutive nights of sampling; however, only data from the 25 females introduced into the enclosure on the first night of the study are included in the analysis of polyandry, because it is only for these females that we have a lifetime record. The total number of matings and number of mating partners of free-living female *G. sigillatus* is shown in Figure 1. Females lived, on average, 21.0 ± 1.31 days (mean ± SE;
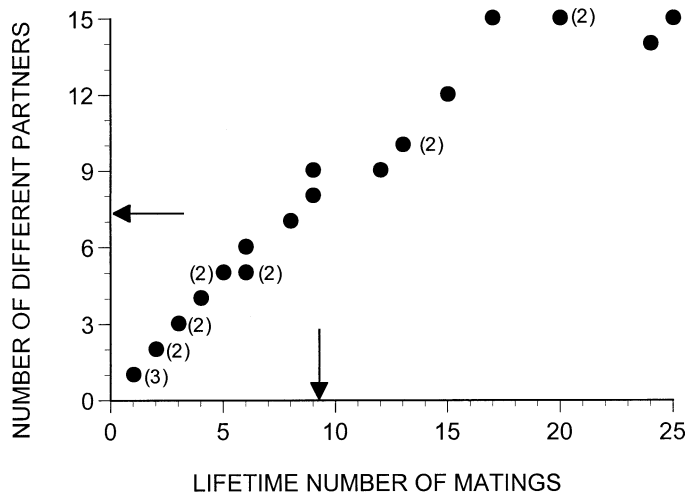
FIG. 1. Lifetime mating frequency and number of different mating partners of 25 free-living female decorated crickets. Means are marked by arrows.

range = 10–31 days). Female mated, on average, 9.20 ± 1.48 times (range = 1–25), with 7.28 ± 0.98 different mating partners (range = 1–15). Female mating frequency and number of different mating partners were positively correlated with female longevity (mating frequency: $r = 0.45$, $P = 0.0245$; number of partners: $r = 0.53$, $P = 0.0065$).

Females frequently mated more than once on the same evening. Taking each night that a female mated as a single observation, 71.5% (118 of 165) of all such female-mating-nights involved single matings, 20% (33 of 165) involved double matings on the same night, 6.1% (10 of 165) involved triple matings, and 2.4% (4 of 165) involved cases in which females mated four times on the same evening. The majority of females (16 of 25) experienced at least one multiple-mating night, and some (11 of 25) experienced two or more. On only two occasions did multiple-mating females ever remate with the same male on the same night, representing $< 5\%$ of all multiple matings.

Offspring Production and Mean Egg Mass of Experimental Females

There were no significant differences in mean offspring production of experimental females across mating treatments ($F = 2.04$, df = 2, $P = 0.14$; Table 1), nor were there any significant differences in offspring production among sibships (i.e., among triads; $F = 0.98$, df = 23, $P = 0.51$). There were also no significant differences in mean egg mass across mating treatments ($F = 0.83$, df = 2, $P = 0.44$; Table 1), nor were there any significant differences in mean egg mass among sibships ($F = 1.10$, df = 23, $P = 0.38$). There was a small, but statistically significant correlation between

female body mass and mean egg mass pooled across all treatments ($N = 72$, $r = 0.318$, $P = 0.0065$). There were no differences in female body mass across treatments ($F = 2.23$, df = 2, $P = 0.12$; Table 1).

Offspring Fitness

There was no significant effect of female mating treatment on the developmental time of offspring, measured as either the time from hatching until the first offspring had undergone adult eclosion ($F = 2.04$, $P = 0.14$), or as the time from hatching until 50% of all surviving offspring had undergone adult eclosion (Table 2, Fig. 2). The nutritional regimen under which offspring were reared had a pronounced effect on both measures of developmental time; under food stress, the time until the first offspring eclosed ($N = 68$, mean = 42.57 days, SE = 0.50) was significantly longer than when offspring were fed ad libitum ($N = 71$, mean = 38.41 days, SE = 0.62; $F = 26.27$, $P < 0.0001$), and so too was the time for 50% of the surviving offspring to eclose significantly longer under food stress (Table 2, Fig. 2). There was a small, but statistically significant interaction between nutritional regimen and mating treatment on the time for 50% of the offspring to eclose (Table 2, Fig. 2); separate analyses of treatment effects within nutritional regimens failed to identify the source of the interaction.

There was no significant effect of female mating treatment on the percentage of offspring surviving to sexual maturity, but there was a significant effect of nutritional regimen on offspring survival (Table 2, Fig. 2). Specifically, a significantly greater percentage of offspring that were fed ad libitum survived to adulthood than did offspring reared under food stress (Fig. 2). There was no significant interaction between nutritional regimen and mating treatment in their effects on offspring survival.

Maximum-likelihood analysis using the CATMOD module of SAS (Stokes et al. 2000) was employed to determine if the sex of offspring at eclosion was independent of nutritional regimen and mating treatment. None of the interactions between the three main effects (sex, nutrition, treatment) were significant (all $P > 0.05$). However, there was a significantly male-biased sex ratio across all nutrition $\times$ treatment levels ($\chi^2 = 12.77$, df = 1, $P = 0.0004$); the proportion of males ranged from 0.502 to 0.590.

There was a significant effect of mating treatment on the body mass of sons, but no significant effect of mating treatment on the body mass of daughters (Table 2, Fig. 3). Post hoc pairwise comparisons between treatments (Tukey's HSD test) revealed that the sons of females given full choice of mates attained a larger body mass than the sons of females given no choice of mates ($P < 0.05$). The sons of females given limited choice of mates also attained larger body mass than the sons of females given no choice, but the difference

TABLE 1. Mean ($\pm$SE) offspring production, egg mass, and body mass of female decorated crickets (ranges shown in parentheses).

Treatment	Number of offspring	Mean egg mass (μg)	Female body mass (mg)
No choice ($N = 24$)	288.1 ± 32.5 (65–563)	218.8 ± 2.8 (196–259)	359.5 ± 11.0 (274–471)
Limited choice ($N = 24$)	225.8 ± 33.6 (52–746)	214.8 ± 2.1 (189–241)	355.5 ± 11.7 (245–471)
Full choice ($N = 24$)	318.0 ± 32.2 (74–616)	215.1 ± 2.5 (185–235)	375.9 ± 10.8 (296–509)

TABLE 2. Effect of dam's mating treatment and offspring nutritional regimen on offspring fitness in decorated crickets.

Variable	Source	df	MS	F	P
Developmental time (50%)	Mating treatment	2	6.3	0.12	0.8877
	Nutritional regimen	1	1196.8	42.53	<0.0001
	Treatment × nutrition	2	72.2	3.62	0.0354
Survival to adulthood	Mating treatment	2	362.5	0.66	0.5234
	Nutritional regimen	1	32580.2	60.06	<0.0001
	Treatment × nutrition	2	20.1	0.04	0.9629
Mass at eclosion					
Males	Mating treatment	2	2109.2	4.52	0.0173
	Nutritional regimen	1	11723.7	18.89	0.0003
	Treatment × nutrition	2	148.0	0.33	0.7202
Females	Mating treatment	2	34.4	0.03	0.9750
	Nutritional regimen	1	18560.0	21.98	0.0002
	Treatment × nutrition	2	1401.5	1.26	0.2970

fell short of statistical significance (95% confidence limits for difference between means: −2.39 to 21.20 mg). There was no significant difference in the body mass of the sons of females given full choice of mates and that of the sons of females given limited choice ($P > 0.05$). Nutritional regimen had a significant influence on the body mass of offspring of both sexes, with offspring fed ad libitum attaining a greater adult body mass than offspring reared under food stress (Table 2, Fig. 3). There was no significant interaction between mating treatment and nutritional regimen in their effects on body mass of offspring in either sex.

DISCUSSION

Free-living female *G. sigillatus* appeared to be continuously receptive, mating repeatedly throughout their life span, a result that mirrors those obtained in the laboratory for this and other cricket species (e.g., Sakaluk and Cade 1980; Solymar and Cade 1990; Burpee and Sakaluk 1993a). Numerous studies have revealed that female crickets derive direct benefits through multiple matings irrespective of whether these
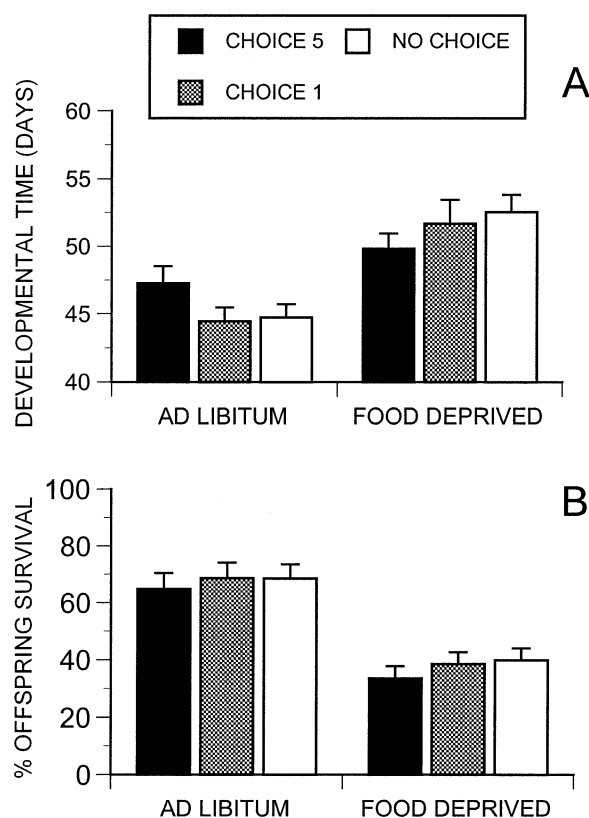

FIG. 2. The effect of female mating treatment on (A) offspring developmental time (time from hatching until 50% of all surviving offspring had eclosed) and (B) percent of offspring surviving to sexual maturity in decorated crickets. Means ± SE are shown.
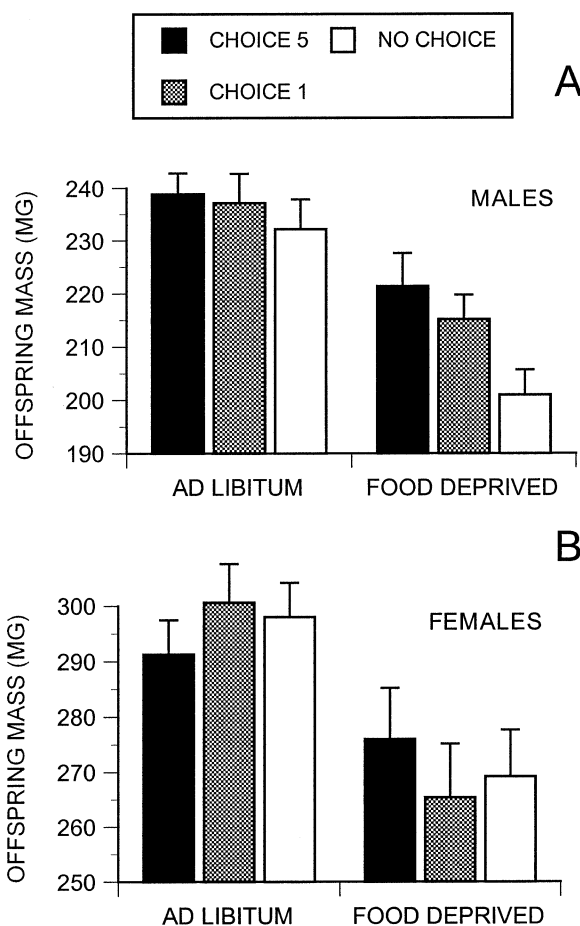

FIG. 3. The effect of female mating treatment on (A) adult body mass of sons and (B) adult body mass of daughters in decorated crickets. Means ± SE are shown.

involve the same or different males (Sakaluk and Cade 1980, 1983; Simmons 1988; Burpee and Sakaluk 1993b; Wagner et al. 2001; but see Tregenza and Wedell 1998). Direct benefits of multiple mating accrue either from substances contained in ejaculates that influence female egg production and oviposition (Destephano and Brady 1977; Loher 1979; Murtaugh and Denlinger 1987), nutrients derived from the consumption of sperm ampullae (Simmons 1988), or both. It seems likely, therefore, that the evolution of the high levels of multiple mating observed in female *G. sigillatus* can be attributed, at least in part, to the direct benefits they obtain from males.

Female *G. sigillatus* also exhibited a high degree of polyandry, mating on average with more than seven different males during their lifetime and up to as many as 15. The apparent ease with which females were able to find additional mating partners was almost certainly facilitated by the tendency of males to aggregate within shelters; because females need not travel widely to find new mating partners, any risk of predation that females incur through phonotaxis to singing males (see Sakaluk and Belwood 1984) is consequently minimized. The levels of polyandry documented in the present study are probably underestimates because, although the majority of matings occur at night, when observations were made, laboratory studies have revealed that 30–40% of all matings can occur during the day (Burpee and Sakaluk 1993a). Crickets had ready access to food and water, but they were also vulnerable to predation by both insectivorous birds and various terrestrial predators. The average longevity of females in the enclosure was comparable to that of crickets collected in the wild (reviewed in Zuk and Simmons 1997), as determined by incremental changes in the cuticle brought about by the daily deposition of chitin (Zuk 1987). Thus, the degree of polyandry observed in this study cannot be attributed to an unusually extended female life span.

Although females exhibited a high degree of polyandry in the outdoor enclosure, the benefits to offspring of polyandry in the laboratory were decidedly modest, at least compared to the effects of the nutritional regimen under which offspring were reared. No differences in offspring survival or development time emerged across mating treatments. However, sons of polyandrous males attained significantly greater body mass than those of monandrous females, although no such difference was detected in daughters.

The apparent asymmetry in the effects of female mating treatment on the mass of male and female offspring would seem to suggest a genetic benefit accruing to female polyandry, but we cannot rule out the possibility of direct benefits. Although the three treatments differed in the number of males with which females had the opportunity to mate, they also may have differed in the overall number of female matings. However, we would expect that any differences in the level of material benefits by females across treatments would be more evident when females were food stressed than when they were provided with food ad libitum. Contrary to this expectation, there was no significant interaction between mating treatment and offspring nutritional regimen in their effects on male offspring mass, suggesting that benefits accruing to female polyandry are independent of the environment in which offspring develop. There were also no significant

differences in mean egg mass across mating treatments, which would appear to rule out the possibility that females differentially allocate resources to their eggs according to their opportunities to mate with preferred males. We cannot, however, rule out qualitative differences in egg provisioning across treatments.

Although we did not monitor the mating activity of females in the three treatments, it is almost certain that females of the limited-choice and full-choice treatments mated with most, if not all, of the males that were available. Previous time-lapse video studies of male-female pairs monitored over five consecutive days have shown that females mate, on average, 12 times in this period and up to as many as 25 (Burpee and Sakaluk 1993a,b; Calos and Sakaluk 1998). Even in the full-choice treatment, it is inconceivable that a single male would have monopolized all of these matings, particularly in light of the observation that females in the enclosure rarely mated twice with the same male in a night. Males require more than 3 h in which to replenish spent spermatophores (Sakaluk 1985), whereas females will remate much sooner than this interval when given the opportunity to do so (Sakaluk 1987). Indeed, in a study in which single females were paired with two males in a mating chamber for 4 h, females mating twice in the same observation period always mated with both males (Sakaluk 1987). Unlike other crickets, male *G. sigillatus* do not engage in physical aggression, so even a particularly robust male would have been unable to prevent other males from mating in the full-choice treatment. Collectively, these observations point to a high degree of polyandry within the limited-choice and full-choice treatments, commensurate with what was observed in the enclosure study.

The sexual difference in the extent to which male and female offspring benefit from female polyandry is consistent with a recent suggestion that any assessment of the benefits of mate choice should account for differences in the fitness returns from sons and daughters (Kokko 2001). The benefits of mate choice, and by extension polyandry, may be manifest more in the subsequent mating success of offspring than in their immediate viability. In decorated crickets, larger sons almost certainly experience a higher reproductive success because the larger nuptial gifts synthesized by these males result in greater sperm transfer (Sakaluk 1984, 1985) and higher paternity (Sakaluk and Eggert 1996); larger males also live longer (Burpee and Sakaluk 1993b). In contrast, large body mass appears to be less important to the reproductive success of daughters because body mass is correlated with neither female fecundity, nor longevity (Will and Sakaluk 1994). In other cricket species, larger males may enjoy increased mating success owing to their success in agonistic interactions with smaller opponents (e.g., Dixon and Cade 1986; Souroukis and Cade 1993; Hack 1997) and their increased attractiveness to females (e.g., Brown et al. 1996; Gray 1997; Bateman et al. 2001). A recent study has shown that male crickets successful in reproduction competition with other males, also sire sons that are more likely to succeed, although this effect was unrelated to male size (Wedell and Tregenza 1999). Thus, polyandry by females may lead to an increase in their sons' reproductive success, a possibility supported by recent work on *Tribolium* (Bernasconi and Kel-

ler 2001; Pai and Yan 2002). Moreover, a sex difference in the extent to which male and female offspring benefit via their mother's polyandry is not unprecedented, having recently been documented in bulb mites (Konior et al. 2001) and flour beetles (Pai and Yan 2002).

If the direct benefits of multiple matings in female crickets are well established, genetic benefits to polyandry are less so, particularly the relative importance of short-term benefits (e.g., avoidance of genetic incompatibility) and long-term effects (e.g., life-history attributes of offspring). In arguably one of the best controlled studies of genetic benefits to date, Tregenza and Wedell (1998) compared the fecundity, hatching success of eggs, and fitness of offspring of female *Gryllus bimaculatus* each mated four times to varying numbers of mating partners (one, two, and four, respectively). There were no differences in fecundity or proportion of eggs across treatments, but the hatching success of eggs increased with the number of mates, providing evidence of short-term benefits to polyandry. There was no evidence of any long-term genetic benefits to offspring because there were no differences in offspring survival, development, and mass at eclosion across treatments. These results seemingly contradict earlier work conducted on this same species (Simmons 1987), albeit using a different experimental design. In Simmons's (1987) study, groups of five females were initially housed with 10 males for a 24-h period, but were held individually thereafter. These females mated, on average, 13.4 times and can essentially be regarded as polyandrous because it is highly unlikely that any given female always mated with the same male. The reproductive output of these females was compared with that of monandrous females randomly assigned either a single, small male or a single, large male for the same 24-h period. Although this experiment does not control for number of matings, monandrous females actually mated more often, on average, than polyandrous females (18.2 vs. 13.4 matings); thus, any reproductive advantage gained by polyandrous females cannot be attributed to greater receipt of direct benefits. In contrast to Tregenza and Wedell's (1998) study, there was no difference in the hatching success of eggs of monandrous and polyandrous females, but polyandrous females exhibited higher fecundity and also laid a greater proportion of their eggs. Although there was no evidence of short-term gains to offspring, there was evidence of significant long-term genetic benefits, as offspring of polyandrous females developed more quickly, and therefore reproduced sooner, than offspring of monandrous females.

Variation in the nature of the genetic benefits derived through polyandry seen in *G. bimaculatus* are mirrored by studies of other cricket species. Simmons (2001) reported a greater egg hatching success of female *Teleogryllus oceanicus* mated once to each of two different males compared to females mated twice to the same male, suggesting short-term benefits to polyandry, but no effect on offspring fitness, survival, development, or offspring mass at eclosion. The present study offers some evidence of a long-term benefit to offspring, at least with respect to offspring mass, but the lack of a difference in the number of offspring produced argues against any short-term gains in *G. sigillatus*.

How do we resolve the apparent inconsistencies in both the level and form of the direct and indirect benefits to poly-andry observed in crickets? One of the striking features of the majority of studies of female multiple mating in crickets (whether with the same or different males) is that experimental levels of the number of matings and/or the number of mating partners are probably much lower than those that females experience in nature. Early studies of female multiple mating examined female reproductive success at the extremes of female frequency, comparing the reproductive success of females mating once versus those mating twice (Sakaluk and Cade 1980) or comparing the reproductive success of females mated once or twice to those given unlimited opportunities to mate (Sakaluk and Cade 1983; Simmons 1988). However, numerous studies have revealed that females of a variety of cricket species mate repeatedly throughout their lives when given the opportunity to do so (e.g., Simmons 1988; Solymar and Cade 1990; Cade and Cade 1992; Burpee and Sakaluk 1993a). A more definitive test of the effect of repeated mating on female reproductive success would entail experimental manipulation of the mating rate of females, while permitting them to mate continuously throughout their lives.

If the propensity for multiple matings by female crickets is well documented, the extent to which such matings involve different males is not. In those studies that have assessed the benefits of polyandry while controlling for mating frequency, females have been permitted to mate with a maximum of only two to four different males (e.g., Tregenza and Wedell 1998, 2002; Simmons 2001; Wagner et al. 2001). However, if the data on polyandry of free-living *G. sigillatus* are at all representative of other multiply mating species, these experimentally established levels of polyandry probably fall far below the level of polyandry in nature. It is, perhaps, no coincidence that the study providing the best evidence of long-term genetic benefits of polyandry to offspring (Simmons 1987) is the one that afforded females the opportunity to mate with the largest number of different males (up to 10). Long-term genetic benefits may only become manifest when females have the opportunity to select among a large number of males (or their sperm; see Eberhard 1996), because the opportunity of encountering a high-quality mate increases with the degree of polyandry. In contrast, it may take matings with only two or three males to correct for the possibility of mating with a genetically incompatible male. Alternatively, just as there appears to be an optimal mating rate in female insects (Arnqvist and Nilsson 2000), there may also be an optimal degree of polyandry beyond which female fitness slowly declines. If that were indeed the case, studies that established different degrees of polyandry might yield apparently contradictory results. What is needed to distinguish among these various possibilities are experiments in which the degree of polyandry is varied across the entire range of polyandry observed under natural conditions; this, in turn, requires more detailed information on levels of polyandry of free-living female insects than is presently available.

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